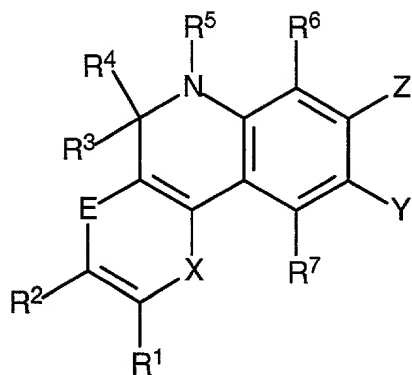


We claim

1. A compound of the formula



wherein

R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or which ring is substituted by -L-R<sub>x</sub> or -L-S<sub>C</sub>;

or R<sup>2</sup> in combination with R<sup>3</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>3</sup> and R<sup>4</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>3</sup> in combination with R<sup>4</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

R<sup>6</sup> is H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>7</sup> is hydrogen, alkyl having 1-6 carbons, or alkoxy having 1-6 carbons; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>, and the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>; and

R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

Y is H, OH, NH<sub>2</sub>, NO, or -(CO)-R<sup>9</sup>, or -(CO)-O-R<sup>10</sup>, where R<sup>9</sup> and R<sup>10</sup> are H, C<sub>1</sub>-C<sub>6</sub> alkyl, or a substituted or unsubstituted aryl or heteroaryl ring system having 1-2 rings;

Z is H, OH,  $\text{NHR}^{17}$ , SH, or  $\text{C}(\text{CR}^{11}\text{R}^{12})_2\text{OH}$ ; where  $\text{R}^{17}$  is a  $\text{C}_1\text{-C}_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen;  $\text{R}^{11}$  and  $\text{R}^{12}$  are independently  $\text{C}_1\text{-C}_6$  alkyls that are optionally substituted by carboxylic acid, sulfonic acid, or halogen, or  $\text{R}^{11}$  and  $\text{R}^{12}$  taken in combination form a 5- or 6-membered alicyclic ring;

5

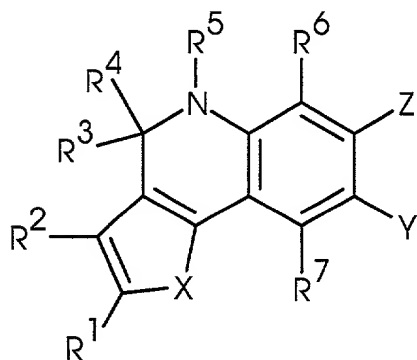
wherein L is a covalent linkage;

$\text{R}_x$  is a reactive group; and

10  $\text{S}_c$  is a conjugated substance.

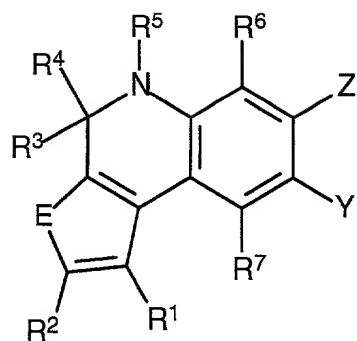
2. A compound, as claimed in Claim 1, wherein one of X and E is O, S, or  $\text{CR}^{1'}=\text{CR}^{2'}$ , and the other is absent.

15 3. A compound, as claimed in Claim 1, having the formula



wherein X is O or S.

20 4. A compound, as claimed in Claim 1, having the formula



wherein E is O or S.

5 5. A compound, as claimed in Claim 2, wherein X is S.

6. A compound as claimed in Claim 1, wherein

R<sup>1</sup> is H or sulfonic acid;

R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>6</sup> and R<sup>7</sup> are each hydrogen or methyl; and

Z is OH.

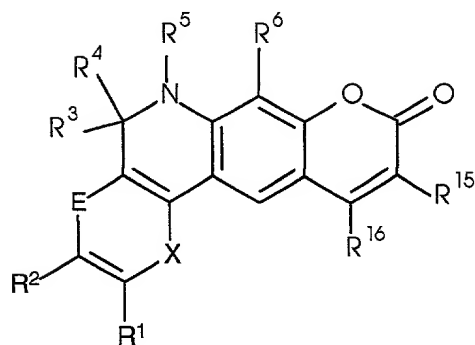
7. A compound, as claimed in Claim 1, wherein Y is H or -(CO)-H or NO.

8. A compound, as claimed in Claim 1, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.

9. A compound, as claimed in Claim 1, wherein  $R_x$  is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

10. A compound, as claimed in Claim 1, wherein  $S_c$  is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

11. A compound of the formula



$R^1$ ,  $R^2$ , and  $R^6$  are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a  $C_1$ - $C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_C$ ;

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

$R^3$  and  $R^4$  are independently H,  $C_1$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_C$ ;

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

$R^5$  is H, methyl, carboxymethyl, a  $C_2$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^5$  is an aryl or heteroaryl ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_C$ ;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

5 one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>; the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>; and

10

R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

15

R<sup>15</sup> and R<sup>16</sup> are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

20

wherein L is a covalent linkage;

25 R<sub>x</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

12. A compound, as claimed in Claim 11, wherein one of X and E is O or S.

30

13. A compound, as claimed in Claim 12, wherein

R<sup>6</sup> and R<sup>7</sup> are H;

R<sup>9</sup> and R<sup>4</sup> are each methyl;

5

R<sup>1</sup> is H or sulfonic acid;

one of R<sup>15</sup> and R<sup>16</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>, and the other is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl; or cyano;

10

wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds, and

15

wherein R<sub>x</sub>, when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group; and

20

wherein S<sub>C</sub>, when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

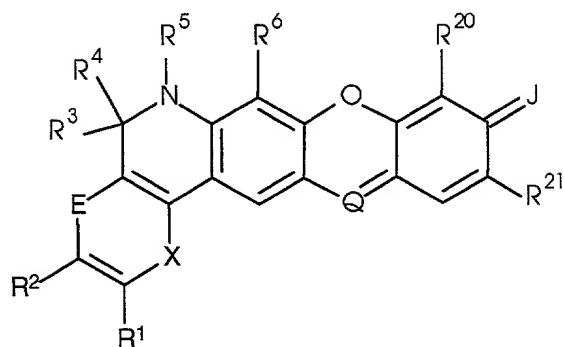
25

14. A compound, as claimed in Claim 11, wherein one of R<sup>15</sup> and R<sup>16</sup> is an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl.

30



15. A compound of the formula



5 wherein

$R^1$ ,  $R^2$ , and  $R^6$  are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a  $C_1$ - $C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_c$ ;

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

$R^3$  and  $R^4$  are independently H,  $C_1$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_c$ ;

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

$R^5$  is H, methyl, carboxymethyl, a  $C_2$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^5$  is an aryl or heteroaryl ring that is optionally

substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or R<sup>5</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>; and the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>; and

R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

R<sup>20</sup> and R<sup>21</sup> are hydrogen, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

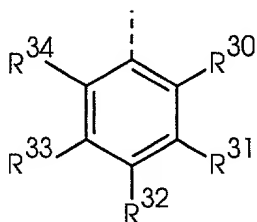
J is O or NR<sup>37</sup>R<sup>38</sup>;

where R<sup>37</sup> and R<sup>38</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; an aryl or heteroaryl ring; or R<sup>37</sup> in combination with R<sup>38</sup> forms a saturated 5- or 6-membered heterocycle that is a piperidine, a morpholine, a pyrrolidine or a piperazine, each of which is optionally substituted by methyl, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alkyl; or

-L-R<sub>x</sub> or -L-S<sub>C</sub>;

or R<sup>37</sup> in combination with R<sup>20</sup>, or R<sup>38</sup> in combination with R<sup>21</sup>, or both, form a 5- or 6-membered ring that is saturated or unsaturated, and is optionally substituted by one or more sulfonic acids, or C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by sulfonic acid;

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> has the formula



where R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, or C<sub>6</sub>-C<sub>18</sub> arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

$S_c$  is a conjugated substance.

16. A compound, as claimed in Claim 15, wherein Q is N.

17. A compound, as claimed in Claim 15, wherein J is O and Q is  $CR^{28}$ .

18. A compound, as claimed in Claim 17, wherein one of  $R^5$ ,  $R^{21}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , and  $R^{34}$  is  $-L-R_x$  or  $-L-S_c$ .

19. A compound, as claimed in Claim 15, wherein

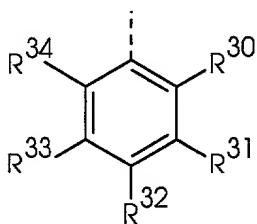
$R^3$  and  $R^4$  are each methyl;

$R^1$  is H or a sulfonic acid;

$R^6$  is H; and

J is  $NR^{37}R^{38}$ .

20. A compound, as claimed in Claim 19, wherein Q has the formula  $CR^{28}$ , wherein  $R^{28}$  has the formula



wherein one of  $R^{30}$ - $R^{34}$  is  $-L-R_x$  or  $-L-S_c$ ; and

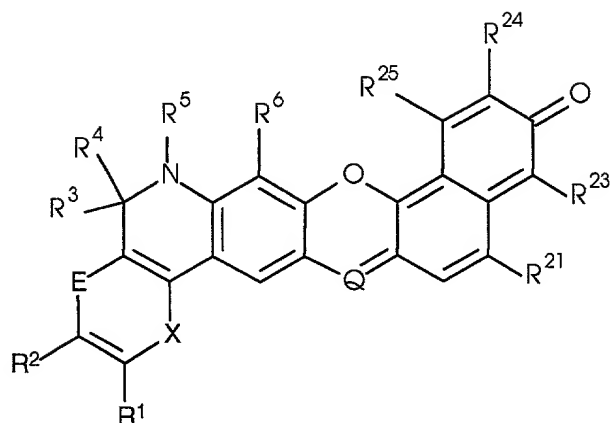
wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds, and

5 wherein  $R_x$ , when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl  
10 halide, or a thiol group; and

wherein  $S_c$ , when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

21. A compound of the formula



5 wherein

$R^1$ ,  $R^2$ , and  $R^6$  are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a  $C_1$ - $C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_G$ ;

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

$R^3$  and  $R^4$  are independently  $C_1$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

5

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>; the other is absent;

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wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

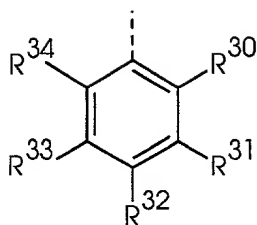
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R<sup>21</sup>, R<sup>23</sup>, R<sup>24</sup>, and R<sup>25</sup> are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

20

25

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> has the formula



where  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or  $C_1$ - $C_{18}$  alkyl,  $C_1$ - $C_{18}$  alkoxy,  $C_1$ - $C_{18}$  alkylthio,  $C_1$ - $C_{18}$  alkanoylamino,  $C_1$ - $C_{18}$  alkylaminocarbonyl,  $C_2$ - $C_{36}$  dialkylaminocarbonyl,  $C_1$ - $C_{18}$  alkyloxycarbonyl, or  $C_6$ - $C_{18}$  arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a  $C_1$ - $C_6$  alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents  $R^{31}$  and  $R^{32}$ ,  $R^{32}$  and  $R^{33}$  or  $R^{33}$  and  $R^{34}$ , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  is -L- $R_x$  or -L- $S_c$ ; and

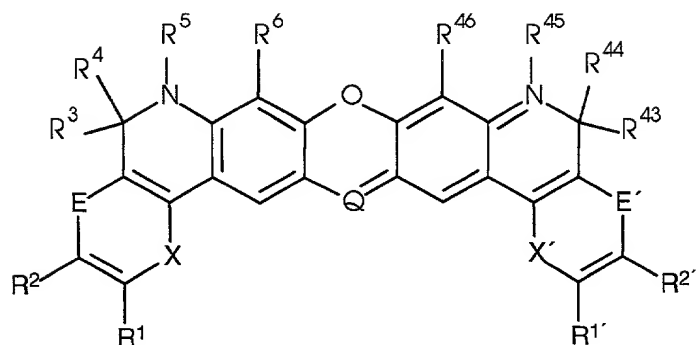
wherein L is a covalent linkage;

$R_x$  is a reactive group; and

$S_c$  is a conjugated substance.



22. A compound having the formula



5 wherein

$R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$ , and  $R^{46}$  are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a  $C_1$ - $C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_C$ ;

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently H,  $C_1$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ ,  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently H, methyl, carboxymethyl, a  $C_2-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^5$  is an aryl or heteroaryl ring that is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

5

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

- 10 one of E and X is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent; and one of E' and X' is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent;

wherein  $R^8$  is H, methyl, carboxymethyl, or a  $C_2-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

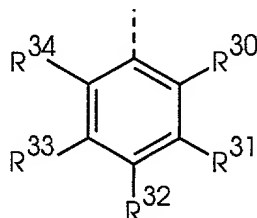
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$R^{1'}$  and  $R^{2'}$  are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a  $C_1-C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

20

Q is N or  $CR^{28}$ , wherein  $R^{28}$  is H, F, CN, carboxylic acid, or a carboxylic acid ester of a  $C_1-C_6$  alcohol; or  $R^{28}$  is a  $C_1-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^{28}$  has the formula

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where  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or  $C_1$ - $C_{18}$  alkyl,  $C_1$ - $C_{18}$  alkoxy,  $C_1$ - $C_{18}$  alkylthio,  $C_1$ - $C_{18}$  alkanoylamino,  $C_1$ - $C_{18}$  alkylaminocarbonyl,  $C_2$ - $C_{36}$  dialkylaminocarbonyl,  $C_1$ - $C_{18}$  alkyloxycarbonyl, or  $C_6$ - $C_{18}$  arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a  $C_1$ - $C_6$  alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents  $R^{31}$  and  $R^{32}$ ,  $R^{32}$  and  $R^{33}$  or  $R^{33}$  and  $R^{34}$ , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  is -L- $R_x$  or -L- $S_c$ ; and

wherein L is a covalent linkage;

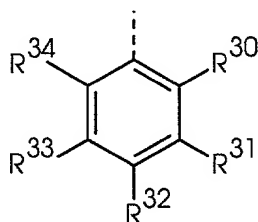
$R_x$  is a reactive group; and

$S_c$  is a conjugated substance.

23. A compound, as claimed in Claim 22, wherein

$X = X'$ ,  $E = E'$ ,  $R^1 = R^{41}$ , and  $R^2 = R^{42}$ .

24. A compound, as claimed in Claim 22, wherein Q has the formula  $CR^{28}$ , and  $R^{28}$  has the formula



25. A compound, as claimed in Claim 24, wherein one of  $R^5$ ,  $R^{21}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ , and  $R^{45}$  is  $-L-R_x$  or  $-L-S_C$ .

26. A compound, as claimed in Claim 24, wherein

5

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are each methyl;

$R^1$  and  $R^{41}$  are independently H or sulfonic acid; and

10  $R^6$  and  $R^{46}$  are H.

27. A compound, as claimed in Claim 24, wherein the compound is substituted one or more times by sulfonic acid.

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28. A compound, as claimed in Claim 22, wherein one of  $R^1$ ,  $R^{1'}$ ,  $R^2$ ,  $R^{2'}$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^{15}$ ,  $R^{16}$ ,  $R^{20}$ ,  $R^{21}$ ,  $R^{23}$ ,  $R^{24}$ ,  $R^{25}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $R^{37}$ ,  $R^{38}$ ,  $R^{41}$ ,  $R^{42}$ ,  $R^{43}$ ,  $R^{44}$ ,  $R^{45}$ , and  $R^{46}$  is an  $-L-R_x$  or  $-L-S_C$ .

20

29. A compound, as claimed in Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.

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30. A compound, as claimed in Claim 28, wherein  $R_x$  is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

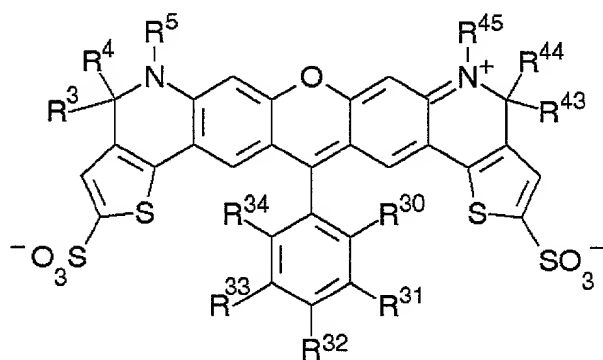
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31. A compound, as claimed in Claim 28, wherein  $R_x$  is a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, or a reactive platinum complex.

32. A compound, as claimed in Claim 28, wherein  $S_c$  is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

33. A compound, as claimed in Claim 28, wherein  $S_c$  is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

34. A compound, as claimed in Claim 28, having the formula:



wherein  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^{43}$ ,  $R^{44}$ , and  $R^{45}$  are independently methyl or ethyl;

$R^{30}$  is sulfonic acid or carboxylic acid;

$R^{31}$  and  $R^{34}$  are independently H, F, or Cl;

one of  $R^{32}$  and  $R^{33}$  is H, F, or Cl, and the other of  $R^{32}$  and  $R^{33}$  is  $-L-R_x$  or  $-L-S_c$ ,

wherein L is a covalent linkage of the formula  $-S(CH_2)_aCOO(CH_2)_b-$  or the formula  $-S(CH_2)_aCONH(CH_2)_b-$

wherein a is an integer between 0 and 10, and b is an integer between 0 and 10 provided that a and b are not both 0; and

wherein  $R_x$ , where present, is a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, or a reactive platinum complex.; and

wherein  $S_c$ , where present, is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

35. A compound, as claimed in Claim 34, wherein  $R_x$  is a maleimide group or is a succinimidyl ester of a carboxylic acid.

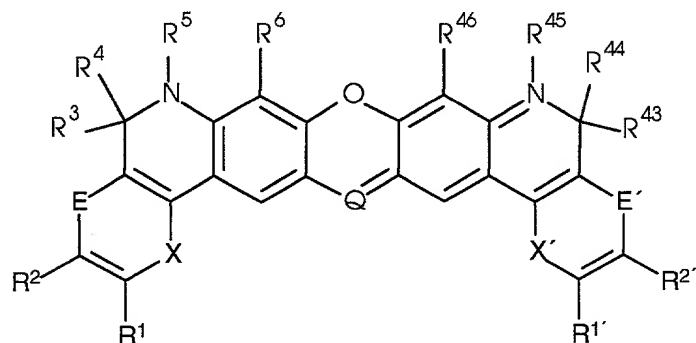
36. A compound, as claimed in Claim 34, wherein  $S_c$  is peptide or a protein or a lectin.

37. A compound, as claimed in Claim 34, wherein  $S_c$  is an antibody or antibody fragment.

38. A compound, as claimed in Claim 34, wherein  $S_c$  is a nucleotide or an oligonucleotide.

39. A compound, as claimed in Claim 34, wherein  $S_c$  is a BAPTA or APTRA ion-complexing moiety.

40. A method of staining a biological sample, comprising:  
combining a dye solution comprising a compound of the formula



wherein

$R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$ , and  $R^{46}$  are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a  $C_1$ - $C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or  $-L-R_x$ ; or  $-L-S_C$ ;

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently H,  $C_1$ - $C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ ,  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently H, methyl, carboxymethyl, a  $C_2-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^5$  is an aryl or heteroaryl ring that is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

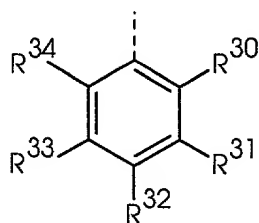
or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent; and one of  $E'$  and  $X'$  is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent;

wherein  $R^8$  is H, methyl, carboxymethyl, or a  $C_2-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a  $C_1-C_6$  alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or  $CR^{28}$ , wherein  $R^{28}$  is H, F, CN, carboxylic acid, or a carboxylic acid ester of a  $C_1-C_6$  alcohol; or  $R^{28}$  is a  $C_1-C_6$  alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^{28}$  has the formula





where  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or  $C_1$ - $C_{18}$  alkyl,  $C_1$ - $C_{18}$  alkoxy,  $C_1$ - $C_{18}$  alkylthio,  $C_1$ - $C_{18}$  alkanoylamino,  $C_1$ - $C_{18}$  alkylaminocarbonyl,  $C_2$ - $C_{36}$  dialkylaminocarbonyl,  $C_1$ - $C_{18}$  alkyloxycarbonyl, or  $C_6$ - $C_{18}$  arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a  $C_1$ - $C_6$  alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents  $R^{31}$  and  $R^{32}$ ,  $R^{32}$  and  $R^{33}$  or  $R^{33}$  and  $R^{34}$ , when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  is -L- $R_x$  or -L- $S_c$ ; and

wherein L is a covalent linkage;

$R_x$  is a reactive group; and

$S_c$  is a conjugated substance;

with a biological sample in a concentration sufficient to yield a detectable optical response under the desired conditions.

41. A method, as claimed in Claim 40, further comprising combining the sample with an additional detection reagent that has spectral properties that are detectably different from said optical response.

42. A method, as claimed in Claim 40, further comprising the step of determining a characteristic of the sample by comparing the optical response with a standard response parameter.

43. A method, as claimed in Claim 40, wherein the sample comprises cells.

44. A method, as claimed in Claim 40, wherein the sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

45. A method, as claimed in Claim 40, further comprising tracing the temporal or spatial location of the optical response within the sample.

46. A method, as claimed in Claim 40, wherein for said compound

at least one of  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $R^{37}$  and  $R^{38}$  is  $-L-R_x$  or  $-L-S_c$ ;

$R_x$  is a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, or a maleimide group; and

$S_c$  is an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, that is optionally bound to one or more additional fluorophores that are the same or different.

47. A method, as claimed in Claim 46, wherein for said compound,  $R^{28}$  is an  $-L-S_c$ , and  $S_c$  is an ion-complexing moiety that is a BAPTA or an APTRA.

48. A method as claimed in Claim 40, wherein at least one of  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $R^{37}$  and  $R^{38}$  is  $-L-S_c$ , and  $S_c$  is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.